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Hematopoietic Stem Cell: Self-renewal versus Differentiation

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Abstract

The mammalian blood system, containing more than ten distinct mature cell types, stands on one specific cell type, hematopoietic stem cell (HSC). Within the system, only HSC possess the ability of both multi-potency and self-renewal. Multi-potency is the ability to differentiate into all functional blood cells. Self-renewal is the ability to give rise to HSC itself without differentiation. Since mature blood cells are predominantly short lived, HSC continuously provide more differentiated progenitors while properly maintaining the HSC pool size properly throughout life by precisely balancing self-renewal and differentiation. Thus, understanding the mechanisms of self-renewal and differentiation of HSC has been a central issue. In this review, we focus on the hierarchical structure of the hematopoietic system, the current understanding of microenvironment and molecular cues regulating self-renewal and differentiation of adult HSC, and the currently emerging systems approaches to understand HSC biology.

Introduction

While mature blood cells are produced at a rate of more than one million cells per second in the adult human [1], most of the hematopoietic stem cells (HSCs) from which they are derived cycle very infrequently and primarily reside in the G_0 phase of the cell cycle under homeostatic conditions [2]. These two facts present an interesting conundrum: how dose the organism achieve a balance whereby an adequate pool of HSCs is maintained for the life of the organism, while at the same time HSCs consistently meet the organism's enormous demand for continuous replenishment of mature blood cells, most of which are short-lived. The importance of this balance is underscored by the numerous examples where aberrant HSC development causes severe disease *e.g.* when HSC differentiation into committed progenitors is not accompanied by the typical loss of self-renewal capacity, or HSC derived progenitors fail to fully differentiate into mature blood cells [3], and may enter a preleukemic progression [4]. These intriguing features of mammalian hematopoiesis have fueled extensive investigation of the system over the last several decades. In this review, we focus on the outlined conundrum, and discuss what is currently known of the regulatory events that govern the ability of HSCs to generate many billions of mature blood cells while at the same time maintaining an adequate pool of HSCs for the entire life of the species.

The Concept of Stem Cells

The "stem cell" concept was first proposed by Till and McCulloch following their pioneering studies of the blood system regeneration *in vivo*. Ten days after transplanting limiting number of syngenic bone marrow (BM) cells into recipient mice, they observed cellular colonies that formed in the spleens of recipient mice. Analysis of these colonies revealed that a very small sub-population of the donor BM cells possessed two remarkable

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properties: (1) the ability to generate multiple types of myeloerythroid cells, and (2) the ability to self-replicate [5–8][1]. These findings introduced the two defining criteria of stem cells *i.e.* multi-potency and self-renewal. Hematopoietic Stem Cells (HSCs) are the only cells within the hematopoietic system that possess the potential for both multi-potency and self-renewal. In the case of HSC, multi-potency is the ability to differentiate into all functional blood cells, while self-renewal is the ability to give rise to identical daughter HSCs without differentiation.

The field of stem cell research has greatly expanded since the initial studies of Till and McCulloch and now includes stem cells that give rise to specific organs/tissues (collectively termed tissue-specific stem cells) and also embryonic stem (ES) cells which can give rise to every cell type in the adult body. A system of nomenclature has evolved to reflect the differentiation potential of different stem cell populations (summarized in Table 1). It is beyond the scope of this article to discuss non-hematopoietic stem cell populations; excellent reviews of the latter cells are presented elsewhere in this issue.

In 1988, the initial prospective purification of hematopoietic stem cells from mouse BM was achieved utilizing the relatively new technologies of multi-color fluorescence-activated cell sorting and monoclonal antibodies [10,11]. The resultant population of enriched mouse HSCs had a surface marker phenotype of Thy-1^{low} Lin (Lineage-markers)$^{-}$ Sca-1^{+}, and represented approximately 0.05% of the mouse adult BM cells. Spangrude and colleagues demonstrated that these were the only cells in mouse BM capable of transferring long-term reconstitution of the entire hematopoietic system (then defined as more than 3 months) when transplanted into lethally irradiated mice [11]. A reductionist approach by Uchida et al showed that Thy1.1^{low}, but not Thy1.1^{high} or Thy1.1^{-} cells could give rise to donor-derived long-term multilineage reconstitution of irradiated hosts; this was true of Sca-1^{+}, but not Sca-1^{-} cells, and of Lin^{-}, but not Lin^{+} cells [12]. Since these initial studies, mouse HSCs have been more extensively purified by identifying and then utilizing additional cell-surface markers to distinguish them from other cells in BM; these included, but were not exclusively single cells that could self-renew and give long-term multilineage maturation [12–14]. In 1994 the population isolated by Spangrude et al was shown to include at least 3 multipotent populations: Long-Term (LT)-HSC, Short-Term (ST)-HSC, and Multi-Potent Progenitor (MPP, a cell population that has lost the self-renewal capacity of HSC) [15]. In 1996, HSCs from adult mouse BM were sufficiently enriched to conduct single-cell transplantation experiments, and these studies revealed that one in three CD34$^{-/low}$ c-Kit^{+} Sca-1^{+} Lin^{-} cells showed myelolymphoid long-term reconstitution in lethally irradiated recipients after a single cell transplant [16]. Despite the fact that hematopoietic tissues contain both stem and progenitor cells, rapid and sustained engraftment of syngenic and even of H2 incompatible allogenic hosts can only be achieved with HSC, the time to engraftment depending on the number of HSC transplanted [17].

Hierarchical Structure of Mouse Hematopoietic System

The mammalian blood system contains more than ten distinct mature cell types including red blood cells (erythrocyte), megakaryocytes/platelets, myeloid cells (monocyte/macrophage and granulocytes), mast cells, T- and B- lymphocytes, natural killer (NK) cells and dendritic cells (DCs). The concept that such diverse cell types are all derived from a common progenitor cell, *i.e.* from HSCs, is intriguing and an indication that HSCs possess

[1]Subsequent studies have shown that the day 10 spleen colonies are derived from committed, multipotent, myeloerythroid progenitors 9. Nakorn, T.N., T. Miyamoto, and I.L. Weissman, *Characterization of mouse clonogenic megakaryocyte progenitors.* Proc Natl Acad Sci U S A, 2003. **100**(1): p. 205-10.. This does not detract from the conceptual origins of the field of stem cell biology by the Till-McCulloch group.

remarkable differentiation potential. To understand how the multi-potent HSC differentiate into these diverse functional cell types, our laboratory and others have used cell surface marker phenotype analysis by flow-cytometry to identify and isolate discrete sub-populations of developing blood cells, together with a battery of well-defined and highly sensitive read-out assays to elucidate their relative functions and differentiation potential. These analyses have suggested a hierarchical structure in hematopoietic development in which multi-potency is progressively restricted (illustrated diagrammatically in Figure 1). HSCs initially give rise to the MPPs which no longer possess self-renewal ability yet keeping full-lineage differentiation potential [15,18]. We now realize that this population of MPPs is still heterogeneous [16,18,19] and additional work to fully understand this heterogeneity is in progress [20–22]. Further downstream, MPPs advance to oligopotent progenitors: 1) the common lymphoid progenitor (CLP) [23–25] and 2) the common myeloid progenitor (CMP) [26]. Collectively these oligopotent progenitors then give rise to all the lineage-committed effector cells of the hematopoietic system *e.g.* CMPs give rise to megakaryocyte/erythrocyte progenitors (MEPs) and granulocyte/macrophage progenitors (GMPs) [9,27]. The position of DCs in this model is interesting. We have shown that the three major phenotypic subsets of these antigen-presenting cells ($CD8\alpha^+$DC, $CD8\alpha^-$DC, and plasmacytoid DC) can be derived either from CMP and CLP [28–30]; the biological functions of the 6 different DC subsets identified in our studies are yet to be elucidated.

Human HSC and Downstream Progenitors

Human HSCs were isolated using similar technologies to those used for mouse HSCs, *i.e.* isolation of cells representing different stages of differentiation on the basis of cell-surface marker phenotype, coupled with functional assays. For human hematopoiesis, the property of long-term reconstitution of the various cell subsets is evaluated in xenotransplantations models, utilizing immuno-deficient mice, sometimes transplanted with fetal human hematolymphoid organs for irradiation-resonstitution assays [31,32]. The first cell-surface marker used to enrich human HSCs was CD34, a ligand for L-selectin that is expressed by only 0.5–5% of blood cells in human fetal liver, cord blood and adult BM [33–35]. *In vitro* assays revealed that almost all $CD34^+$ cells have multi-potency or oligo-potency, but also that the population was still very heterogeneous.

The first prospective isolation of human HSC exhibited the phenotype of $CD34^+CD90^+Lin^-$, where the Lin markers include T, B, NK, and Myeloerythroid specific markers [31]. These cells generated lymphoid and myeloid progeny in both *in vitro* colony assays and *in vivo* SCID (severe combined immunodeficiency)-hu mice; in contrast, the remaining population of $CD34^+CD90^-Lin^-$ cells were unable to generate cell clones containing myeloid and lymphoid cell types [31]. Further enrichment of human HSCs within the $CD34^+$ population was achieved via differential expression of the surface marker CD38. Although most of $CD34^+$ cells (90–99%) co-express CD38, cells that can give rise to multi-lineage colonies containing both lymphoid and myeloid cells reside in the CD38 low to negative fraction and the $CD90^+$ fraction. [31,36–39]. Furthermore, $CD34^+CD38^-$ cells and not $CD34^+CD38^+$ cells are highly enriched for long-term culture-initiating cells [36,40] and contain cells capable of repopulating the absent lymphoid compartment of SCID mice [31] and NOD (non-obese diabetes)-SCID immunodeficient mice [41,42]. Indeed enrichment of human HSCs from G-CSF mobilized peripheral blood on the basis of CD34 and CD90 co-expression has improved the efficiency of human bone marrow transplantation by providing cells that mediated hematopoietic reconstitution and long-term engraftment but which have greatly reduced contamination of unwanted host cancer cells or immunoreactive T cells [43–45]. Virtually all $CD34^+$ $CD90^+$ Lin^- cells reside in the $CD38^-$ fraction [39], leading to the overall conclusion that human HSC are enriched in the Lin^- $CD34^+$ $CD38^-$ $CD90^+$ population of human sources of hematopoietic cells.

While this latter population of Lin^{-} $CD34^{+}$ $CD38^{-}$ $CD90^{+}$ human cells is greatly enriched for human HSCs, retroviral or lentiviral marking of this population prior to xenotransplantation into immunodeficient mice has revealed functional heterogeneity of the cells [46–48]. Our own studies have recently identified a human MPP population in human cord blood defined as Lin^{-} $CD34^{+}$ $CD38^{-}$ $CD90^{-}$ $CD45RA^{-}$ which exhibit the functional properties of multipotency and an incomplete self-renewal capacity [49]. Further downstream of MPP, early myeloid and lymphoid committed progenitors have been identified in the human hematopoietic system that give rise to likely counterparts of murine CMP, GMP, MEP, and CLP [1,50–53]. Using IL-3Ra and CD45RA (an isoform of CD45 that negatively regulates some select classes of cytokine signaling), human CMP, GMP, and MEP populations have been identified and verified through *in vitro* and *in vivo* assays [53].

In both mouse and human hematopoiesis, this complex multi-tiered scheme (see Figure 1) allows for an enormous amplification in the numbers of terminally differentiated cells, while at the same time maintaining precise regulation of stem cell homeostasis

Niche: Essential Microenvironment for HSC

Essential insights into molecular mechanisms regulating HSC self-renewal and differentiation were provided prior to HSC isolation. Investigations of two mast cell-deficient spontaneous mutant mouse strains, the W strain [54], and the Sl strain [55], lead to the concept of a ‘niche’ *i.e.* an essential microenvironment for HSCs. The W gene locus is on chromosome 5, and the Sl gene locus is on chromosome 10. Mutant mice of both W gene and Sl genes show quite similar phenotypes; anemia, lack of mast cells, lack of melanocytes, and lack of germ cells [56–62]. When normal BM cells are transplanted into W mutant mice, the hematopoietic system is completely restored. However, when transplanted into the Sl mutant mice, hematopoiesis is still abnormal. When BM cells from the W mutant mice are transplanted into either wild-type or the Sl mutant mice, hematopoiesis is not corrected. On the other hand, when BM cells of the Sl mutant mice are transplanted into the W mutant mice, hematopoiesis is reconstituted. These observations lead to the proposal of a model that a specific microenvironment or ‘niche’ comprising cells outside the hematopoietic system is required for self-renewal and differentiation of HSCs. The transplantation experiments just outlined were best explained by concluding that the gene on the W locus is essential for functional HSCs and the gene on the Sl locus is essential for some environmental element essential for hematopoiesis but not contained in bone marrow hematopoietic cells *i.e.* so-called “niche” [63–66]. In 1990, the critical gene in the W locus was found to be that encoding cytokine receptor-tyrosine kinase c-Kit [67,68]; at approximately the same time, the important gene in the Sl locus was found to encode steel factor (SLF), also known as kit-ligand or Stem Cell Factor (SCF) [69]. The bone marrow niche for HSC in mice has been partially characterized: Clonal bone marrow stromal cells have been found that support HSC self-renewal and myelo-lymphoid maturation [70]. Phenotypic populations enriched for HSC have been located near the endoosteum [71,72] and near blood vessels in the bone marrow [19]. The bone structures that include bone and HSC niches can be reconstituted ectopically with highly purified fetal bone osteochondral stem/progenitor cells, but not osteogenic progenitors alone [21]. These findings are evidence that the interactions between the receptors and ligands between HSCs and the niche are essential for maintenance of the hematopoietic system.

Molecular cues governing HSC

Although many cytokines and their receptors have been investigated utilizing gene-targeting technology, few receptors/ligands have been shown to play essential roles in HSC function (Table 2, 3). Two such receptor/ligand pairs that are critical for HSC are SCF/c-Kit

(described above), and TPO/c-Mpl. Studies using prospectively isolated mouse HSC have shown that both c-Kit and c-Mpl are expressed on highly purified HSC [13,73–78], and genetic elimination of either TPO or c-Mpl leads to reduction of HSC[75,76]. Consistent with these data, cytokines SCF and TPO can both support survival and proliferation of purified mouse HSC assayed in serum-free culture at the single cell level [79]. TPO signaling of HSC is thought to occur via an association ligand-bound c-Mpl with the adaptor protein Lnk, an intracellular scaffold protein that acts as an inhibitor of many cytokine signaling pathways, including SCF, EPO, IL-3, IL-7 as well as TPO [74,79–82]. Detailed studies of Lnk−/− mice have revealed not only increased absolute numbers of HSC but also increased repopulation potentials of individual HSC in competitive repopulation assays, suggesting that Lnk is a negative regulator of HSC self-renewal [74,79,83]. Moreover, TPO, not SCF, was the responsible signaling pathway for the increased self-renewal capacity of Lnk−/− HSCs.[74,79], a finding consistent with earlier studies that the generation of fetal liver HSC is not impaired in Sl/Sld or W/Wv mice [13]. These studies suggested that TPO signaling is a positive regulator of HSC self-renewal, and Lnk is a key negative regulator of self-renewal signaling of HSC.

In addition to SCF and TPO, the availability of purified HSCs and recombinant proteins has enabled investigation of the direct effects of cytokines on HSC. Functional effects of many cytokines including IL-3, IL-6, IL-11, Flt-3 ligand in combinations with either SCF and/or TPO have been reported. In most cases, exposing HSCs to combinations of these cytokines results in survival and proliferation of cells. However, in most studies, these cells immediately lose long-term repopulation potential as assessed in transplantation assays. Later studies have reported that the Flt-3 receptor is not expressed on HSCs [18,73]. Similarly, the IL-11 receptor knockout mice showed normal hematopoiesis [84], question an essential role for this receptor-ligand system on HSC function. In terms of the latter apparent contraction, it has now become clear that many cytokines have redundant functions at the level of either receptor binding or intracellular signal transduction. Thus further investigation is required, with comprehensive study involving antagonism of multiple related receptors in order to clarify this complex area.

Apart from classical hematopoietic cytokines, developmental signaling pathways have also been shown to be relevant for adult HSC. Purified Wnt3a treatment *in vitro* increases self-renewal of mouse adult HSCs [85,86]. Overexpression of constitutively active β-catenin, an intracellular transducer of Wnt signaling, in mouse HSCs followed by long-term culture results in dramatic expansion of functional HSC as measured by both cell-surface phenotype and transplantation assays. Consistent with these observations, ectopic expression of axin or a frizzled ligand-binding domain, both mediators that inhibit the Wnt signaling pathway, lead to inhibition of HSC growth *in vitro* and reduced constitution activity after transplantation [86]. However, subsequent gain- and loss-of-function studies yielded more complicated results. Two independent studies utilizing *in vivo* conditional expression of β-catenin revealed impaired multilineage differentiation and a transient increase in HSC numbers, followed by exhaustion of the HSC pool [87,88]. Furthermore, opposite approaches to conditionally inactivation of β-catenin in HSCs showed contradictory results with some of these studies showing normal hematopoiesis and HSC activity in the combined absence of β-catenin and its homologue γ-catenin [89–92]. These discrepancies may reflect an elaborate redundancy of Wnt signaling molecules, the presence of compensatory signal transduction pathways, and the complicated dose-response of signal transduction, thus unresolved mechanisms behind the effects remain to be addressed. (For further review: http://www.stanford.edu/~rnusse/wntwindow.html)

In contrast to the high turnover of lineage-restricted progenitors, most of HSCs reside in the ‘quiescent’ G_0 phase of the cell cycle [2]. TGF-β/Smad signaling is one of the responsible

pathways that maintain quiescence of HSCs. *In vitro* culture studies have revealed inhibitory effect of TGF-β on HSC proliferation without inducing apoptosis [93–95]. Moreover, neutralization of TGF-β *in vitro* was shown to release early hematopoietic progenitor cells from quiescence [96–98]. Several molecular mechanisms have been proposed for TGF-β mediated inhibition of HSC proliferation, including alterations in cytokine receptor expression, inhibition of lipid raft clustering which amplifies cytokine signaling, and up-regulation of cyclin-dependent kinase inhibitors *e.g.* p21 and p57 [96,99–109]. However, loss-of-function strategies to elucidate direct effect of TGF signaling on HSC have been challenging. Despite various phenotypes in mature blood cells, conditional knockout mice of the TGF-β type I receptor displayed normal HSC self-renewal and regenerative activity *in vivo* [110], and mice deficient for the TGF-β type II receptor have not been well characterized in the context of HSC function. Furthermore, due to the high redundancy of the Smad molecules (which are the intracellular transducer of TGF signaling), and early embryonic lethality of most Smad and TGF-β knockout mouse models, *in vivo* elucidation of the role of the TGF/Smad signaling pathway in HSC function has proven difficult.

Another proposed cue for HSC quiescence is Ang-1/Tie2. Tie2 is a receptor tyrosine kinase expressed on endothelial cells and HSCs [111–113]. Despite the fact that Tie2 knockout mice models are embryonic lethal, studies utilizing chimeric mice containing normal, Tie1-null, or Tie2-null cells showed that these receptors are not required for prenatal hematopoiesis. However Tie2 is indispensable to maintain the HSC pool in adult BM [114]. Arai et al reported that in physiological conditions, HSCs expressing Tie2 are quiescent and reside next to BM osteoblasts which express Ang-1. Furthermore, *in vitro* Ang-1 treatment suppresses proliferation of HSCs and maintains *in vivo* long-term repopulating activity [115]. These results suggest that Ang-1/Tie2 signaling is an ideal target for modification of HSC quiescence.

Systems Approaches to Understand HSC Biology

The preceding sections summarize information about regulation of HSC fate that has been gained from traditional approaches utilizing gene overexpression, gene deletion, and direct stimulation of highly purified HSC with recombinant proteins. While valuable insights have been gained, these approaches are limited by the lack of demonstration of physiological *in vivo* relevance, including genetic redundancy underlying systems that are essential for host survival, such as the hematopoietic system. For example, a “back-up” gene or pathway can compensate functionally for the deleted gene in a gene knockout mouse in a manner that masks the true physiology of normal homeostasis. Our laboratory and numerous others have focused on a currently emerging alternative approach, termed systems approach, to shed light on the complex molecular network regulating HSC fate. Although the extremely low frequency of HSC in BM makes it difficult to obtain large numbers of pure HSCs, recent advances in microarray technologies have enabled systematic profiling of gene expression by small numbers of purified HSCs. Gene expression profiling studies of highly purified mouse adult HSCs are summarized in Table 4. Microarray analysis detects the concentration of target mRNAs encoded by genes expressed by a cell population by the high stringency hybridization of an mRNA preparation from the cell population to a microarray matrix expressing very large numbers of synthetic oligonucleotide probes. The efficiency of this event depends on target and probe sequences, and so is not uniform for each target mRNA. Based on this limitation, microarray assay provides only differentially expressed genes after comparison of more than 2 samples. When a particular cell population *e.g.* HSCs is compared to two other different cell populations, the list of differentially expressed genes between each cell population pairing will be different. Thus, interpretation of microarray studies depends on both method of the HSC purification, and also the counterpart cell population to which HSCs are being compared.

In 2002, Park et al generated microarrays of 5000 genes selected for early hematopoietic cells by subtracting a large cDNA library generated from lineage-committed hematopoietic cells from a cDNA library generated from purified HSC/MPP populations. They then compared gene expression of a population enriched for HSC (Rho^{low}KSL, $Rhodamine^{low}$ c-Kit^{+} $Sca\text{-}1^{+}$ Lin^{-}) and another enriched for MPP (Rho^{hi}LSK). Throughout these screens, 30 HSC (Rho^{low}KSL) specific genes, 30 MPP (Rho^{hi}LSK) specific genes, and 70 commonly expressed genes were reported [116]. Utilizing Affymetrix type arrays, Ivanova et al compared human fetal liver HSC, LCP (Lineage Committed Progenitor), MBC (Mature Blood Cell); mouse fetal liver HSC, LCP, MBC; and mouse adult LT-HSC, ST-HSC, LCP, MBC. They reported that there were 822 HSC-specific genes conserved between mouse and human. Also, they addressed genetic mechanisms operating in multiple stem cell types by comparing mouse HSC, NSC (neural stem cell), and ES cells, and revealed 283 genes that are commonly expressed among these three stem cell types[117]. The next year, Akashi et al. characterized gene expression patterns according to early lineage commitment by comparing HSC, MPP, CMP, and CLP[118]. Terskikh et al. also characterized expression of 1200 selected mouse genes in 7 early hematopoietic populations (HSC, CMP, MEP, GMP, CLP, pro-T, and pro-B) [78]. These studies revealed successive expression of lineage-specific genes according to hematopoietic hierarchy, and active transcriptional status of HSCs even though they are cell cycle quiescent.

Around 2005, advances in both microarray technology and bioinformatics, such as used in the Gene Ontology project, allowed for further detailed profiling. Forsberg et at. analyzed LT-HSC ($Thy1.1^{low}Flk2^{-}$KSL), ST-HSC ($Thy1.1^{low}FL^{+}$KSL), and MPP ($Thy1.1^{-}Flk2^{+}$KSL) using arrays produced at the Stanford Microarray Facility, covering 42000 genes. From the list of differentially regulated genes, several candidate cell-surface markers were proposed[119]. Later, one of them, the cell adhesion molecule endothelial cell-selective adhesion molecule (Esam1) was shown to be a HSC cell surface marker [120]. To elucidate the mechanisms underlying hematopoietic aging, Rossi et al. compared properties of highly purified HSCs between young mice and old mice by functional assays as well as gene expression profiling. Phenotypic HSC are found in higher frequency in the marrows of 2 years old mice compared to marrows of 2 months old mice [121,122]. They found that in concert with mouse age, HSC increase self-renewal activity and differentiation capacity to generate myeloid lineages, and diminishes lymphoid productivity. Microarray analyses revealed that HSC aging was accompanied by the systemic down-regulation of genes responsible for lymphoid commitment. Moreover, many genes involved in leukemic transformation were up-regulated in HSCs from old mice [122].

Progress in amplification technology has enabled evaluation of gene expression at a single cell level. Ramos et al profiled gene expression of 12 single HSC enriched by sorting with a combination of cell-surface marker expression as $Sca\text{-}1^{+}Gr\text{-}1^{-}$ and differential Hoechst dye efflux ability (Side Population, SP), and reported high diversity of gene expression by these individual cells selected from these population[123]. In 2007, Chambers et al. characterized population enriched for HSC, as defined by SP $Sca1^{+}$ c-Kit^{+} Lin^{-} and terminally differentiated populations including erythrocyte, granulocyte, monocytes, NK cells, activated and naïve T- and B-cells, and published lists of genes uniquely expressed in each cell type[124]. Apart from microarray technology, Yashiro et al. performed high-throughput sequencing of cDNA libraries from two different definitions of HSC, $CD34^{-}$KSL and SP Lin^{-} cells. After digital subtraction to exclude ubiquitously expressed genes, they reported 25 HSC specific genes including five novel genes as well as 29 miRNA-like non-coding RNAs[125].

As seen in Table 4, microarray platforms used have been rapidly evolving within 6 years. At the same time, with continuous progress in the method for HSC purification, each study uses

different criteria to define ‘HSC’, and uses a different counterpart cell population for comparison. Thus, it is not yet possible to cross-compare the lists of differentially regulated genes provided by each study. On the other hand, current accumulation of a number of gene profiling experiments on the same platform enables an exhaustive analysis. Recently, Miranda-Saavedra et al. assembled 271 microarray datasets from 15 published studies that compared gene expression profiles of 37 distinct mouse hematopoietic populations from 15 distinct studies already published, and provided an integrated database to browse these gene expressions named “BloodExpress” (http://hscl.cimr.cam.ac.uk/bloodexpress). This database will be one of the frameworks to integrate our emerging understanding of the kinetics of gene expressions mapped on the entire representation of the cells in the hematopoietic hierarchy[126]. Just as there is as rapid progress of the microarray technology for gene expression profiling, recently emerging methodologies for systems profiling of epigenetic status, microRNA expression, protein expression, and protein phosphorylation are expected to be applicable to purified HSC in the near future. One such analysis of human hematopoietic stem and progenitor cells has resulted in the beginnings of a systems approach to understand human hematopoietic cell biology [127]

Summary

The blood system reflects the balance of two essential abilities of HSC, self-renewal and differentiation. Intensive studies have revealed the hierarchical structure of the blood system and key molecules regulating HSC. However, the entire picture of the molecular interactions orchestrating HSC fate is yet unclear. Synergies between highly developed biological and molecular approaches and rapidly emerging systems approaches will hopefully integrate and accelerate our understanding of this tiny but mighty HSC population.

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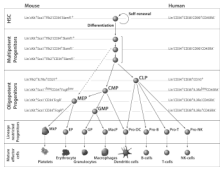

Figure 1. Model of the hematopoietic hierarchy

The HSC resides at the top of the hierarchy, and is defined as the cell that has both the self-renewal capacity and the potential to give rise to all hematopoietic cell types (multi-potency). Throughout differentiation, a HSC first loses self-renewal capacity, then loses lineage potential step-by-step as it commits to become a mature functional cell of a certain lineage. The cell surface phenotype of each population is shown for the mouse and human systems. Intermediate precursors between the first lineage committed progenitors and final mature cell, and different subsets of mature B- and T-cells are omitted. In the mouse system, heterogeneity of MPPs has been revealed by differences in cell surface marker phenotypes and functional differences of their subsets discussed. For example, evidence suggests that some of MPPs directly give rise to MEP without passing through CMP (dashed arrow). HSC: hematopoietic stem cell, CLP: common lymphoid progenitor, CMP: common myeloid progenitor, MEP: Megakaryocyte/erythrocyte progenitor, GMP: granulocyte/macrophage progenitor, MkP: Megakaryocyte progenitor, EP: erythrocyte progenitor, GP: granulocyte progenitor, MacP: macrophage progenitor, DC: dendritic cell, NK: natural killer, Lin: lineage markers.

Table 1

Designations used to Define Differentiation Potential of a Cell Populations.

Designation	Differentiation potential implied by designation	Examples of Stem/Progenitors with these Properties
Toti-potent	All embryonic and extraembryonic tissues	zygote
Pluri-potent	All embryonic tissues	ICM, ES cell, iPS cell
Multi-potent	All lineages of a tissue/organ	HSC, NSC
Oligo-potent	Several but not all lineages of a tissue/organ	CMP, CLP
Uni-potent	Single lineage of a tissue/organ	Macrophage progenitor

Abbreviations used in Table 1: ICM: inner cell mass, ES: embryonic stem, iPS: induced pluripotent stem, HSC: hematopoietic stem cell, NSC: neural stem cell, CMP: common myeloid progenitor, CLP: common lymphoid progenitor

Table 2

Phenotypes of Cytokine KO mice

Cytokine	Receptor	Fetal Development	Phenotype on Blood System in Adult	Number of HSC in adult	Ref
SCF*	c-kit	Normal	Macrocytic anemia	Decrease	See text
TPO	c-Mpl	Normal	Thrombocytopenia	Decrease	[128]
Ang-1	Tie-2	Lethal (E 12.5)	N/A	See text	[129]
IL-3	IL3Ra + Csf2rb	Normal	Lack of response to infection in Mast Cells	Normal	[130]
IL-6	IL6R + Il6st	Normal	Decrease of T-cells in peripheral blood	Normal	[131]
IL-11	Il11Ra + Il6st	No report of KO mice			–
TGF-b1	TGFbr1 + TGFbr2	Lethal (E 10.5) ~ Normal	Significant increase of monocyte & neutrophil		[132,133]
TGF-b2	TGFbr1 + TGFbr2	Born with multiple defects	Not evaluated	Not evaluated	[134]
TGF-b3	TGFbr1 + TGFbr2	Born with abnormal lung development and cleft palate	Not evaluated	Not evaluated	[135]

* SI mutant mice

N/A: not applicable

Table 3

Phenotypes of Receptor KO mice

Receptor	Fetal Development	Phenotype on Blood System in Adult	Number of HSC in Adult	Ref
c-Kit*	Lethal (postimplantation) ~ Normal	Macrocytic anemia	Decrease	See text
c-Mpl	Normal	Thrombocytopenia	Decrease	[136]
Tie-2	Lethal (E 10.5)	N/A	N/A	[137,138]
Il3Ra	Normal	Normal	Normal	[139]
Csf2rb	Normal	Acidocytopenia	Normal	[140]
Il6st (gp130)	Lethal (E 12.5~term)	N/A	N/A	[141]
Il11Ra	Normal	Normal	Normal	[84]
Tgfbr1	Lethal (E 10.5)	N/A	See text	[142]
Tgfbr2	Lethal (E 10.5)	N/A	N/A	[143]

*W mutant mice

N/A: not applicable

Table 4

Studies of Mouse Adult HSC Gene Expression Profiling

Year	Microarray platform	HSC	Compare to	Ref
2002	Homemade	$Rho^{low}KLS$	$Rho^{hi}KLS$	[116]
2002	Affymetrix MU-U74-2 A~C	Rho^{low} KLS	Various type of cells	[117]
2003	Affymetrix MU-U74-2 A~B	$Thy1.1^{low}$ Rho^{low} KLS	MPP CMP CLP	[118]
2003	Clontech Atlas Mouse cDNA array	$Thy1.1^{low}$ KLS	CMP CLP GMP MEP ProT ProB	[78]
2005	Stanford Micoarray Facility 42k mouse cDNA array	$Thy1.1^{low}$ $Flk2^{-}$ KLS	$Thy1.1^{low}$ $Flk2^{+}$ KLS $Thy1.1^{-}$ $Flk2^{+}$ KLS	[119]
2005	Affymetrix Mouse 430 2.0	$CD34^{-}FL^{-}KLS$ (Young)	$CD34^{-}FL^{-}KLS$ (Old)	[122]
2006	Affymetrix Mouse U74A	SP $Sca1^{+}$ $Gr1^{-}$	CD8 T-cell	[123]
2007	Affymetrix Mouse 430 2.0	SP $Sca1^{+}$ $c\text{-}Kit^{+}$ Lin−	Erythrocyte Granulocyte Native T Activated T B cell Monocyte NK	[124]
2008	High throughput sequencing and Digital subtraction	$CD34^{-}$ KSL and SP Lin^{-}		[125]

Abbreviations used in this table; Rho: Rhodamine, KLS: $c\text{-}kit^{+}$ Lin^{-} $Sca\text{-}1^{+}$, SP: Side Population